

1. (Thrice Amended) A set of promoter sequences suitable for optimizing the expression of a gene in a selected organism or group of organisms, said set of promoter sequences covering a range of promoter activities for said gene in small steps each step changing the activity by 50-100%, each promoter sequence of said set of promoter sequences comprising a double stranded DNA sequence, the sense strands of which comprise

at least two consensus sequences, said at least two consensus sequences corresponding to conserved sequences identified in said organism or group of organisms, at least half of each of said consensus sequences being kept constant in the set of promoter sequences, the at least two consensus sequences, when the selected organism or group of organisms is prokaryotic, being selected from the group consisting of TATAAT and TTGACA, when the selected organism or group of organisms is eukaryotic, being selected from the group consisting of a TATA-box and a UAS upstream of said TATA-box and, between said consensus sequences or flanking at least one of said consensus sequences, at least one nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied by random incorporation of nucleotides that are selected from the group consisting of the nucleobases A, T, C and G.

16. (Thrice Amended) A method of constructing a set of promoter sequences which is suitable for optimizing the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences, which consensus sequences correspond to conserved sequences identified in said organism or group of organisms, at least one of the consensus sequences being flanked by a non-conserved nucleotide spacer sequence or both or said consensus sequences

being separated by the non-conserved nucleotide spacer sequence, the at least two consensus sequences, when the selected organism or group of organisms is prokaryotic, being selected from the group consisting of TATAAT, TTGACA and an activator binding site upstream of the TATAAT sequence, when the selected organism or group of organisms is eukaryotic, being selected from the group consisting of a TATA-box and a UAS upstream of said TATA-box,

SUB H1 cont
F2 cont
(ii) constructing a set of single stranded DNA sequences each of which comprises at least half of each of the consensus sequences, and a non-conserved nucleotide spacer sequence, at least part of which is varied by a random incorporation of nucleotides selected from the group consisting of the nucleobases A, T, C and G, whilst keeping the at least half of the consensus sequences constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences to obtain the set of promoter sequences covering a range of promoter activities for said gene.

F3
18. (Four Times Amended) A method of controlling in an organism the flux of a cellular metabolite or the expression of a desired gene product, said method comprising at least one step of changing the expression level of at least one gene in the pathway leading to formation of said metabolite or the expression level of said desired gene product, the step comprising

SUB H1
(i) selecting from the set of promoter sequences of claim 1 a plurality of promoter sequences covering a range of promoter activities for said gene, in steps, each step changing the β -galactosidase activity by 50-100%,

(ii) transforming said set of promoter sequences into cells of the organism, placing in each of said cells the gene to be expressed under the control of at least one promoter of the set,

SUB HI
cost
F3
conew

(iii) cultivating the transformed cells to obtain clones thereof and selecting among said clones a clone having, relative to an otherwise identical clone where the at least one gene in the pathway or the gene expressing the desired gene product is under the control of its native promoter, a higher or a lower flux of the cellular metabolite or a higher or a lower expression of the desired gene product.

F4

21. (Four Times Amended) A method of isolating a promoter sequence being capable of optimizing the expression of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16, a set of promoters covering a range of promoter activities for said gene, in steps, each step changing the B-galactosidase activity by 50-100%,

(ii) transforming said set of promoters into cells of the selected organism, placing in each of said cells the gene to be expressed under the control of at least one promoter of the set,

SUB HI

(iii) cultivating the transformed cells to obtain clones thereof and selecting among said clones a clone having, relative to an otherwise identical clone where the at least one gene in the pathway or the gene expressing the desired gene product is under the control of its native promoter, a higher or a lower flux of the cellular metabolite or a higher or a lower expression of the desired gene product, and

(iv) isolating said promoter sequence from the clone.

F5
SUB HI

23. (Amended) A set of promoters according to claim 1 suitable for optimizing the expression of a gene in a prokaryotic organism wherein the promoter